You discover that the dirt in your neighbors basement has the ability to initiate neurons to start growing after they have stopped for a long period of time. Using separation chromatography, you find that a 4-catecholamine derivative complex is responsible. Your scientific curiosity pushes you to want to know everything about this molecule, and how it has this very useful ability to initiate neuronal regeneration. For the purpose of this exam, we will call this small magical molecule "RCS137". Using ligand affinity chromatography, you determine that this molecule binds to a protein in neurons which you have named "neuronase".

1. (25 points) You first want to examine neuronase by a variety of biophysical techniques to avoid solution/gel artifacts. By solution transport techniques (light scattering, sedimentation, etc.) you find that the protein behaves as a 174 kD protein (1kD equals 1000 g/mol). When neuronase is run on an SDS-PAGE gel, it migrates alongside a marker equal to 29 kD. You then measure the osmotic pressure of neuronase at 37 °C using a membrane with a molecular weight cut-off (MWCO) of 1,000 g/mol. In a solution of 8 mg/mL protein in dilute buffer, you find the osmotic pressure to be 1.17×10^{-3} atm.

(a) (10 points) Explain the above results. What is the molecular weight of neuronase? How does it exist in solution?

$\pi = RT\frac{w}{M} \Rightarrow M = \frac{RTw}{\pi} = \frac{\left(.0820 \frac{L\cdot atm}{K\cdot mol}\right)(310K)\left(8 \frac{g}{L}\right)}{(1.17 \times 10^{-3} atm)}$

$= 1.74 \times 10^5 \frac{g}{mol}$ or 174 kD

MW of neuronase is 29 kD if runs as a monomer on the SDS gel. In soln it exists as a hexamer of MW = 174 kD. $\frac{174 kD}{29 kD} = 6$

(b) (15 points) List 5 molecular properties that describe the protein. How would you obtain each property listed?

1	MW	$M = \frac{RTs}{D\cdot(1-\bar{\nu}\rho)}$ or $M = \frac{RTw}{\pi}$	comb. of sedimentation and diffusion expts → osmotic pressure meas. w/ osmometer
2	D	$D = \frac{kT}{f}$	light scattering
3	V, r	$V = \frac{M}{N_0}(\bar{\nu}_2)$ $r = \left(\frac{3}{4\pi} V\right)^{1/3}$	r from x-ray crystallography
4	$\bar{\nu}$	$\frac{1}{\rho}$	band sedimentation
5	Ze	$Ze = \mu f$	electrophoresis

2. (25 points)

(a) (15 points) You perform a sedimentation experiment and determine that neuronase neither sinks nor floats in a solution of density 0.8 g/cm^3. What is the partial specific volume v_2 of neuronase? Assuming that neuronase is an unsolvated sphere of mass m, determine the volume, radius r, and frictional coefficient f_o for the molecule. The viscosity of the medium η may be assumed to be that of water, 1 x 10^{-2} g s^{-1} cm^{-1}.

$\bar{v}_2 = \frac{1}{\rho} = \boxed{1.25 \frac{cm^3}{g}}$ $\quad m = \frac{M}{N_0} = \frac{174{,}000 \frac{g}{mol}}{6.02 \times 10^{23} \frac{molec.}{mol}} = 2.89 \times 10^{-19} \frac{g}{molec.}$

$V = m \bar{v}_2 = (2.89 \times 10^{-19} g)(1.25 \frac{cm^3}{g})$

$\boxed{V = 3.61 \times 10^{-19} cm^3}$

$V = \frac{4}{3}\pi r^3 \Rightarrow r = (\frac{3}{4\pi} V)^{1/3} = \boxed{4.41 \times 10^{-7} cm}$

$f_o = 6\pi\eta r = 6\pi (.01 \frac{g}{s\,cm})(4.41 \times 10^{-7} cm) = 8.31 \times 10^{-8} \frac{g}{s}$

(b) (5 points) You determine the diffusion coefficient D for neuronase in dilute buffer at 37 °C to be 6.47 x 10^{-8} cm^2/s. Calculate the frictional coefficient f.

$D = \frac{kT}{f} \Rightarrow f = \frac{kT}{D} = \frac{(1.38 \times 10^{-16} \frac{g\,cm^2}{s^2\,K})(310 K)}{6.47 \times 10^{-8} \frac{cm^2}{s}}$

$f = 6.61 \times 10^{-7} \frac{g}{s}$

(c) (5 points) What is the ratio f/f_o ? Assuming that the difference between the values f and f_o is not due to hydration, what is another possible reason for the discrepancy?

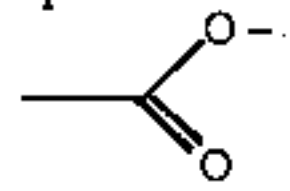
$\frac{f}{f_o} = \frac{6.61 \times 10^{-7} \frac{g}{s}}{8.31 \times 10^{-8} \frac{g}{s}} \cong 8$

large discrepancy could be due to shape, e.g. the f for a very long thin molecule would not be well approximated at all by the assumption that its volume was spherical.

Note: In reality this ratio is more like 1-2.

3. (25 points)
(a) (6 points) Using ligand affinity chromatography, you found that RCS137 binds to the positively charged neuronase. You believe that a particular aspartate residue in neuronase is important for binding to RCS137. You decide to test this theory by mutating the aspartate to an asparagine. What effect will this mutation have on the electrophoretic mobility of neuronase versus the wild-type (non-mutated) form:

aspartate:



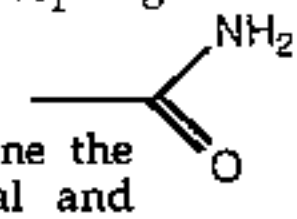
(i) when run on an SDS-PAGE gel?

no change - MW is the same

(ii) when run on an isoelectric focusing gel?

μ of mutant will be different from the μ of the wild type since they have different charge.

asparagine:



(b) (19 points) You perform an equilibrium dialysis experiment to examine the binding of RCS137 to neuronase, which you believe to have six identical and independent binding sites. You measure the osmotic pressure of a solution of neuronase to be 3.0×10^{-3} atm at 37 °C, using an osmometer. You then place the neuronase solution into a dialysis bag and measure the binding of RCS137 to neuronase by equilibrium dialysis at the same temperature. In one run, after binding equilibria is established, the concentration of RCS137 outside the bag is 1.0×10^{-4}, and the total concentration of RCS137 inside the bag is 4.5×10^{-4}.

(i) (10 points) What is the equilibrium binding constant K for RCS137–neuronase binding in these conditions?

RCS137: $c(\text{bound, inside}) = c(\text{total, inside}) - c(\text{outside})$
$= 4.5 \times 10^{-4} - 1 \times 10^{-4} = 3.5 \times 10^{-4}\,M$

Neuronase: $\pi = RTc \Rightarrow c = \frac{\pi}{RT} = \frac{3 \times 10^{-3}\,atm}{\left(0.08206 \frac{L \cdot atm}{K \cdot mol}\right)(310\,K)} = 1.18 \times 10^{-4}\,M$

$$\nu = \frac{c(\text{RCS, bound})}{c(\text{neuronase})} = \frac{3.5 \times 10^{-4}\,M}{1.18 \times 10^{-4}\,M} \cong 3$$

$$K = \frac{\nu}{[\text{RCS137}](N - \nu)} = \frac{3}{(1 \times 10^{-4}\,M)(6-3)} = 1 \times 10^{4}\,M^{-1}$$

(ii) (9 points) You repeat your equilibrium dialysis experiment in the same conditions as above using your mutant neuronase, and find that K for the mutant neuronase is 200 times greater than the K for wild-type. Compute the change in ΔG of binding for neuronase caused by the mutation.

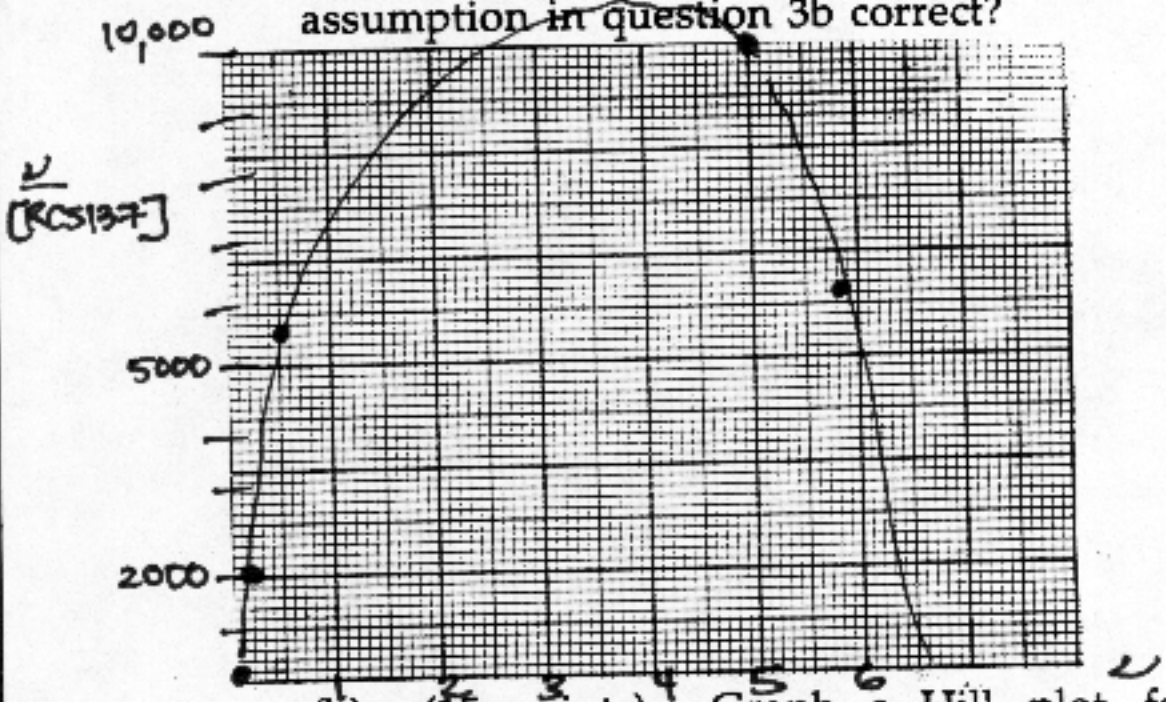
$$\Delta\Delta G^\circ = \Delta G^\circ_{mut} - \Delta G^\circ_{wt}$$
$$= -RT \ln K_{mut} - (-RT \ln K_{wt})$$
$$= -RT \ln \frac{K_{mut}}{K_{wt}} = -\left(8.3145 \frac{J}{K \cdot mol}\right)(310\,K) \ln 200$$

$$\Delta\Delta G^\circ = -1.37 \times 10^{4} \frac{J}{mol}$$

4. (25 points)
For the wild-type neuronase, you repeat the equilibrium dialysis experiment at different concentrations of RCS137. The values you obtain for v, the average number of RCS137 molecules bound to neuronase, at the different RCS137 concentrations are as given in the data table below. [RCS137] is given in g/L.

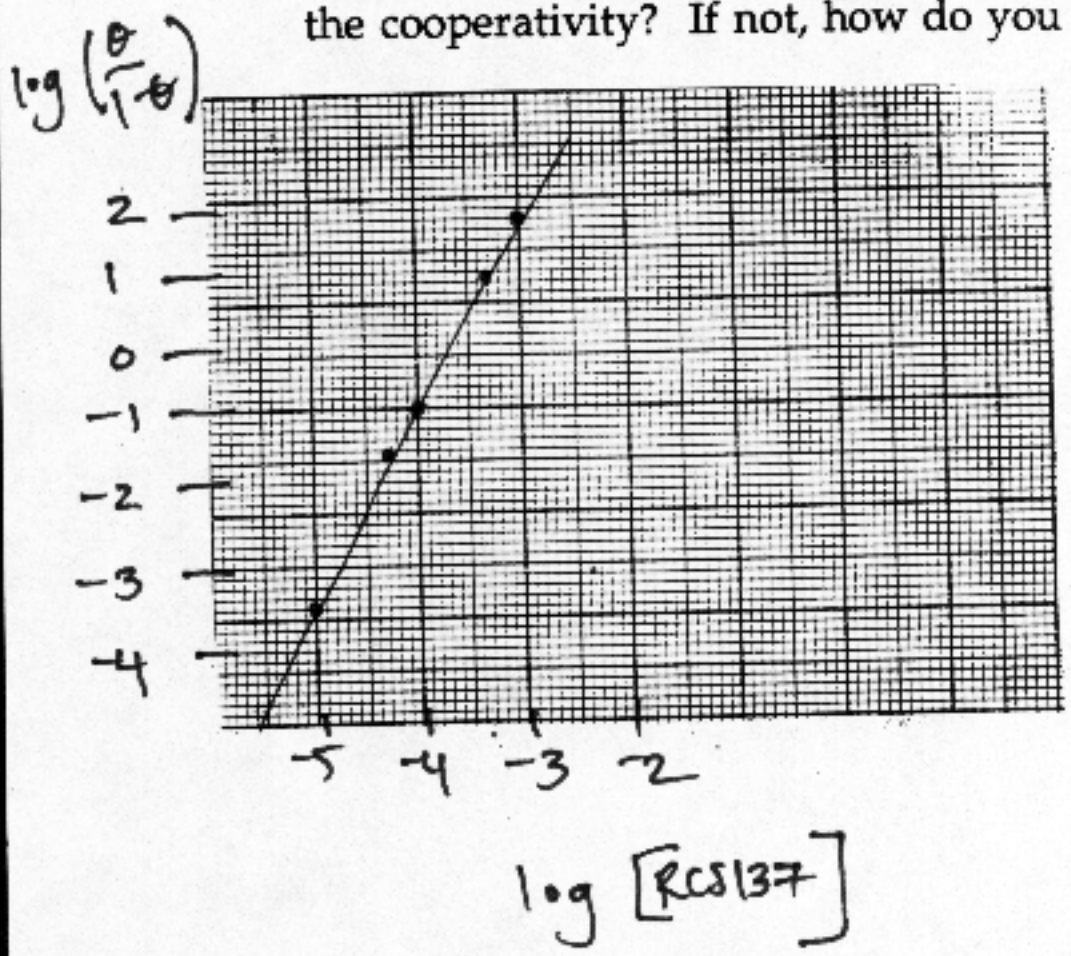
	log [RCS137]	log {v/N/(1-v/N)}	[RCS137]	v/N/(1-v/N)	v/N	v	v/[RCS137]
0	-5.0000	-3.5000	1.0000e-05	0.00031623	0.00031613	0.0018968	189.68
1	-4.3010	-1.7500	5.0000e-05	0.017783	0.017472	0.10483	2096.7
2	-4.0000	-1.0000	1.0000e-04	0.10000	0.090909	0.54545	5454.5
3	-3.3010	0.75000	0.00050000	5.6234	0.84902	5.0941	10188
4	-3.0000	1.5000	0.0010000	31.623	0.96935	5.8161	5816.1

(a) (10 points) Graph a Scatchard plot for the RCS137–neuronase binding. What does the plot tell you about the nature of the neuronase binding sites? Was your assumption ~~in question~~ 3b correct?



Scatchard plot indicates that the binding sites are not independent or identical, hence the assumption in 3b was not correct.

(b) (15 points) Graph a Hill plot for the RCS137–neuronase binding. Is RCS137–neuronase binding cooperative? If so, what can you say about the nature of the cooperativity? If not, how do you know?



slope is ~ 2.5.

∴ binding is positively cooperative.

If binding were not cooperative, the slope of the Hill plot would = 1; $0 < n < 1$ would indicate negative cooperativity.